A COMPARISON OF THE CURVES OF LIPOLYTIC ACTIVITY AND PROTEOLYSIS OF CERTAIN ACID-FAST BACILLI IN NUTRIENT BROTHS

STUDIES IN ACID-FAST BACTERIA. X*

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The demonstration of an active lipase in solution in broth cultures of certain rapidly growing human tubercle bacilli, the curve of lipolytic activity of which, measured in terms of liberation of acid from ethyl-butyrate, follows rather closely their curve of protein metabolism,[1] suggests that this lipase may play a part in the preparation of certain nutritional substances for assimilation by these bacteria. There is a certain amount of presumptive evidence in favor of this view, for the lipase appears to be active even after the organisms are removed from the media in which it is developed, and it is most abundant or most active when bacterial development is intense. Furthermore, this lipase acts on a variety of esters and glycerids,[2] which fact, altho not proving its exogenous functions, suggests that it is sufficiently versatile in its attack to split those substances of a fatty nature it might be confronted with. If this lipase is indeed a true exoferment, it might be justifiable to assume that other bacteria of the same type as the tubercle bacillus (other acid bacteria) would also elaborate such a ferment.

The observations recorded here indicate that filtrates of broth cultures of certain acid-fast bacilli, other than the human tubercle bacillus, do exhibit such lipolytic activity.

The organisms studied in this connection were the so-called leprosy bacillus (Duval), the grass bacillus, and the smegma bacillus. The first organism is of unknown history; the last two were received from Professor Winslow of the American Museum of Natural History. The technical details of measuring the lipolytic activity of such cultures has been described in detail previously,[3] and will not be repeated here.

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1. See preceding article.
2. Study VIII.
3. Study VI.

TABLE 1
LIPOLYTIC ACTIVITY OF SMEGMA BACILLUS

Days	Plain Broth				Dextrose Broth			
	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	—0.50	9.1	2.83	0.00	—0.90	8.4	2.61	0.10
3	—1.10	0.7	0.22	0.15	—1.10	7.0	2.2	0.60
6	....	....	....	....	—1.90	5.6	1.74	1.15
10	—1.50	11.2	3.48	0.60	—1.10	18.9	5.87	1.75
15	—1.50	19.6	6.08	1.30	—1.10	22.4	6.96	1.75
21	—1.60	7.7	2.39	0.80	—2.10	28.0	8.70	1.95
28	—1.40	7.7	2.39	0.50	—2.10	20.3	6.30	1.00
36	—1.30	0.00	0.00	0.50	—1.20	23.8	7.40	1.10
43	—1.80	4.2	1.30	0.60	—2.40	18.2	5.66	1.60
52	....	....	....	....	—2.20	—7.7	—2.39	1.30

TABLE 2
LIPOLYTIC ACTIVITY OF GRASS BACILLUS III

Days	Plain Broth				Dextrose Broth			
	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	—0.10	0.7	0.22	0.05	—0.70	9.8	3.04	0.20
3	—0.20	1.4	0.44	0.20	—0.90	0.7	0.22	0.40
6	—1.10	4.2	1.30	0.55	—1.80	0.7	0.22	0.75
10	—1.70	14.7	4.57	0.60	—1.60	5.6	1.74	1.40
15	—1.50	24.5	7.60	1.60	—2.00	23.8	7.40	1.75
21	—2.00	28.3	7.40	1.80	—2.20	21.7	6.75	1.65
28	—1.60	20.3	6.30	0.90	—2.20	16.1	5.00	0.80
36	—1.90	7.0	2.20	1.00	—2.30	8.4	2.60	1.05
43	—1.80	1.4	0.44	1.10	—2.40	1.4	0.44	1.15
52	—2.00	—4.9	—1.52	1.30	—2.10	—4.9	—1.52	1.15

TABLE 3
LIPOLYTIC ACTIVITY OF DUVAL'S LEPROSY BACILLUS

Days	Plain Broth				Dextrose Broth			
	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{Total\ N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	0.00	0.00	0.00	0.05	—0.50	—0.7	—0.22	0.00
3	—0.20	0.00	0.00	0.05	—0.60	—1.4	—0.44	0.10
10	—1.20	0.00	0.00	0.05	—1.20	0.00	0.00	0.00
21	—0.70	0.70	0.22	0.20	—1.00	—1.4	—0.44	0.00
28	—1.50	—2.8	—0.87	....	—0.90	—12.6	—3.92	0.20
43	—2.20	—12.6	—3.92	0.20	—2.20	—16.8	—5.20	0.10
52	—1.90	—11.9	—3.70	0.10	—2.20	—14.7	—4.56	0.10

TABLE 1—(*Continued*)
LIPOLYTIC ACTIVITY OF SMEGMA BACILLUS

	Mannite Broth				Glycerin Broth			
Days	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	—0.30	8.4	2.60	—0.05	—0.30	—0.7	—0.22	0.05
3	—0.70	0.00	0.00	0.05	—0.20	—0.7	—0.22	0.05
6	—1.30	0.00	0.00	0.10	—0.50	4.2	1.30	0.95
10	—0.80	1.4	0.44	1.75	—0.50	—2.8	—0.87	1.10
15	—0.50	16.8	5.22	1.20	—0.30	2.8	0.87	1.10
21	—0.90	16.8	5.22	1.60	—0.60	12.6	3.91	1.55
28	—1.50	2.80	8.70	1.10	—0.60	14.0	4.40	1.80
36	—1.80	14.0	4.35	1.00	—0.60	0.7	0.22	1.05
43	—1.60	19.6	6.04	1.65	0.00	0.7	0.22	1.75
52	—2.00	—5.6	—1.74	1.30	—0.40	—4.9	—1.52	1.40

TABLE 2—(*Continued*)
LIPOLYTIC ACTIVITY OF GRASS BACILLUS III

	Mannite Broth				Glycerin Broth			
Days	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	—0.40	9.8	3.04	0.10	—0.10	7.7	2.39	0.20
3	—0.40	0.00	0.00	0.15	—0.30	0.00	0.00	0.25
6	—0.70	0.7	0.22	0.75	—1.00	0.00	0.00	0.05
10	—0.90	1.4	0.44	1.60	—0.40	7.7	2.39	1.00
15	—1.10	8.4	2.60	1.80	—0.30	13.3	4.13	1.70
21	—1.30	15.4	4.78	1.80	—0.30	13.3	4.13	1.60
28	—1.30	11.9	3.70	1.30	—0.30	16.8	5.22	1.20
36	—1.40	16.1	5.00	1.40	0.10	15.4	4.78	1.10
43	—1.80	2.8	0.88	1.60	0.00	4.9	1.52	1.05
52	—1.80	—4.9	—1.52	1.30	—0.20	1.4	4.30	1.25

TABLE 3—(*Continued*)
LIPOLYTIC ACTIVITY OF DUVAL'S LEPROSY BACILLUS

	Mannite Broth				Glycerin Broth			
Days	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH	Phenol-phthalein	NH_3 mg. Increase per 100 c.c. Broth	$\frac{NH_3}{\text{Total } N_2}$ Percent	Ethyl-butyrate c.c. N/50 NaOH
1	—0.10	9.8	+3.04	0.00	0.00	1.4	0.44	0.10
3	—0.30	0.00	0.00	0.05	—0.10	1.4	0.44	0.10
10	—0.80	—4.9	—1.52	0.00	—0.50	—0.7	—0.22	0.05
21	—0.90	—4.9	—1.52	0.00	—0.80	—5.6	—1.74	0.05
28	—0.60	—9.8	—3.04	....	—1.30	—9.1	—2.82	....
43	—1.40	—6.3	—1.95	0.05	—1.30	—11.2	—3.48	0.10
52	....	....	....	....	—1.70	—13.3	—4.13	0.10

The tables indicate that, with the exception of the Duval organism, broth cultures of these acid-fast bacteria do exhibit lipolytic activity; furthermore, there is a tendency for the maximum period of proteolysis to coincide with the period of maximum lipolytic activity. The organisms, however, present certain peculiarities in their metabolism curves which deserve further consideration before their exact significance is set forth, and for this reason only the most general statements are warranted.

TABLE 4

THE INITIAL TOTAL NITROGEN AND THE RESIDUAL TOTAL NITROGEN OF VARIOUS MEDIA AFTER THE ORGANISMS HAD GROWN IN THEM FOR 52 DAYS

	Smegma Bacillus	Lepra Bacillus	Grass Bacillus
Plain broth—			
Initial total N_2—mg.	—	322	322
Final total N_2—mg.	—	266	231
Loss total N_2—mg.	—	56	91
*Percent loss	—	17.4	28.3
Dextrose broth—			
Initial total N_2—mg.	322	322	322
Final total N_2—mg.	224	266	231
Loss total N_2—mg.	98	56	91
*Percent loss	30.4	17.4	28.3
Mannite broth—			
Initial total N_2—mg.	322	—	322
Final total N_2—mg.	238	—	245
Loss total N_2—mg.	84	—	77
*Percent loss	26.1	—	23.9
Glycerin broth—			
Initial total N_2—mg.	322	322	322
Final total N_2—mg.	301	112	266
Loss total N_2—mg.	21	210	56
*Percent loss	6.51	6.52	17.4

* Percent loss = percentage of initial total nitrogen which has been appropriated into the bacteria as they have increased in the medium. This nitrogen fraction is subject to variations, increasing as growth increases, and decreasing as vegetative activity wanes and autolytic processes cause partial solution of the bacterial cells.

The Duval bacillus reacts quite differently from the others; altho it grew luxuriantly in all the media, the lipase activity demonstrable in culture appears to be practically nil. Further observations with this organism are in progress.

An approximate measure of the luxuriance of growth of these bacteria, at any stage of the history of the culture, may be obtained by determining the amount of nitrogen which has been tied up in their bodies incidental to their growth. This is readily accomplished by comparing the total nitrogen of the uninoculated medium with that of the clear medium underlying the firm, tenacious pellicle of bacteria which have grown in media of the same composition. Table 4 shows the initial total nitrogen and the residual total nitrogen in milligrams

per 100 c.c. of various media after the organisms had grown in them for fifty-two days. (These bacteria were grown in media of 100 c.c. volume.)

In general, with the exception of the "lepra bacillus," the acid-fast bacteria discussed show a parallelism in their curves of lipolytic activity, as measured by the liberation of acid from ethylbutyrate and their curves of proteolysis. This parallelism is discernible in cultures in plain, dextrose, mannite, and glycerin broths.